Intraoperative Fluorescence Surgical Goggle

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Abstract

Background

Tumor surgery is a great challenge to surgeons. Due to their disseminated characteristic, the small and varied foci and blurred margins often evade surgeon's unaided eyes. Unfortunately, surgeons have to rely mainly on preoperative images and their experience. No effective method is available that can provide real-time reference. Based on targeted fluorescence contrast agents, we are developing an intraoperative imaging system (surgical goggle) for identifying locations and margins of tumors.

Method

Tumor targeted MuCC49-Cy7 is injected into a female athymic nude mice. We use light at a near infrared (NIR) range (710 – 760nm) to excite the fluorophore. An ICG filter (810-875nm) is used to collect fluorescence from MuCC49-Cy7 in vivo. Also, non-fluorescence image is acquired immediately after the fluorescence image with the illumination of light at longer wavelength. Two images are processed, merged then sent to a Head Mounted Display (HMD, vision goggle) for virtual reality display. To validate the accuracy of the system, we use surgical goggle to perform mice anatomy and visualize fluorescence in vitro.

Result

The emission fluorescence is not detectable with bare eyes since it is in the NIR range that provides deeper penetration. However, with the help of surgical goggle, we are able to clearly identify locations and margins of tumors on the mice. At the 1st day after IV injection of MuCC49-Cy7, we can visualize dispersive fluorescence. At the 3rd day after the injection, fluorescence mainly accumulates at tumor sites. Mice anatomy validates that the merged im-

age correctly delineate tumors.

Conclusion

Our surgical goggle can provide real-time, intraoperative reference during surgery. It is a robust and reliable method for segmenting and subsegmenting tumor.

Introduction

Accurate assessment of surgical resection margins and recognition of occult metastatic disease are important oncologic principles in cancer surgery. [1, 2]Currently utilized modalities of cancer detection and imaging focus primarily on preoperative image acquisition and may not specifically target the cancer cell environment. While preoperative imaging is helpful, it fails to provide the surgeon with real-time, cancer-specific information that may be critical for decision-making in the operating room. The ultimate need to bridge these gaps in cancer detection and imaging remains a significant challenge for cancer surgeons, and once overcome, such advancements will ultimately revolutionize the intraoperative applications of the cancer-specific technologies for cancer surgery.

We are developing a wearable surgical navigation system for intraoperative detection of resection margin and occult disease during cancer surgery. Major function is that the surgical navigation system with tumor-specific antibody-fluorophore conjugate is able to provide the

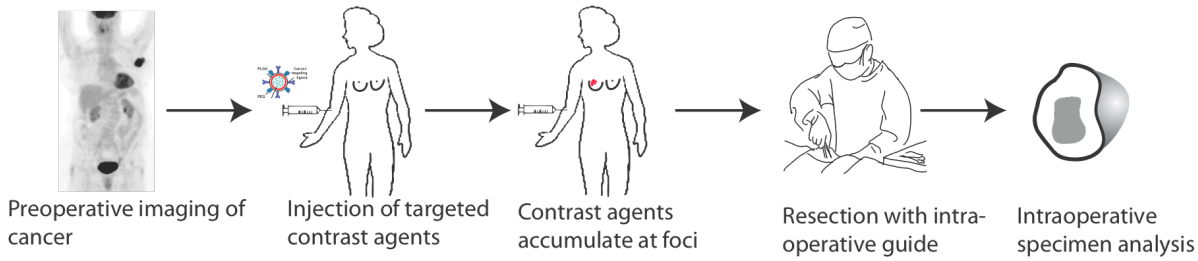

Fig. 1 Process of intraoperative guidance tumor surgery

surgeon with intraoperative, real-time information to accurately assess surgical resection margins and precisely locate occult disease. Also, the surgeons can also assess the resection specimen intraoperatively. This system is tested on phantoms and animal models.

Method

1. Disease markers: *HuCC49ΔC$_H$2 antibody*

Several biomolecular markers are available for cancer target. As an example, Mono-Colonal antibodies (trastuzumab, etc) target tissue markers and involve in tumor growth and metastasization (EGFR, C-erB-2, VEGF). It is reported that ECD HER2-neu, p53 and nucleophosmin antibodies seem to be suitable candidates for different associations. [3] These are used for breast cancer target. For different cancers, there is always a specialized over-expression and corresponding antibody for targeting. We uses CC49, an antibody against tumor-associated glycoprotein, TAG-72 which is an antigen expressed on the majority of human adenocarcinomas, such as breast, ovarian, colorectal and other carcinomas, as our disease marker. In previous studies, TAG-72 targeted HuCC49C$_H$2-Cy7 is fabricated by conjugating humanized CC49 monoclonal antibody (HuCC49C$_H$2) with Cy7 and tested it on female athymic nude mice with colorectal tumors[4]. We tested our HuCC49C$_H$2-ICG as biomarker for tumor detection.

2. Imaging markers: HuCC49ΔC$_H$2 with ICG derivative

CC49 is an antibody which against tumor-associated glycoprotein, TAG-72, an antigen expressed on the majority of human adenocarci-

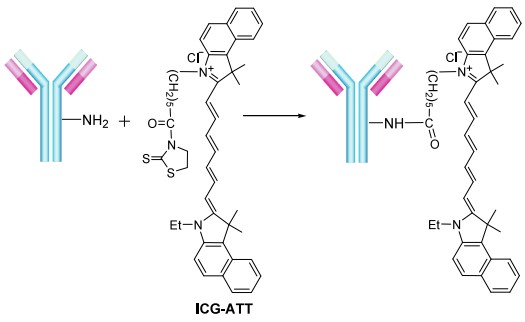

Fig. 2 HuCC49ΔC$_H$2-ICG derivative conjugate

nomas, such as breast, ovarian, colorectal, and other carcinomas.

ICG-ATT is an FDA approved fluorescent dye ICG derivative with strong absorption at 765 nm[5-8]. As shown in **Fig. 2**, HuCC49ΔC$_H$2 is readily labeled with ICG-ATT by acylating the primary amino group of the antibody with optimal molar ratio of ICG-ATT to antibody (8:1) to give maximum fluorescence [6]. ICG-ATT (0.5 mg) will be prepared in 200µl dimethylformamide (DMF) and gradually added to the antibody solution with stirring for 90 minutes. The conjugated antibody will be purified with a PD-10 column. Absorbance of labeled antibody will be measured at 786 nm to determine the concentration of the solution. We conjugate Cy7 with the disease-specific biomarker. Cy7 is a derivative of ICG with an absorption peak at 730 nm. The peak falls into the near infrared wavelength range, enabling thick tissue imaging and tomography. Our colleague has successfully fabricated and published the result[4].

3. Hardware and software platform

Fig. 3 shows hardware design of our prototypic system. It is built on a vision goggle platform (Personal Cinema System, Headplay Canada Inc). Superbright LEDs (Super Bright LEDs Inc.) are integrated with the goggle or used as handheld probe. RGB camera (QuickCam® Pro 9000, Logitech) is installed on the vision goggle. A long pass filter on the camera collects fluorescence while eliminating interference from the excitation light. Also, non-fluorescence background image is acquired to provide background reference. The system is driven by the National Instrument DAQ-6210.

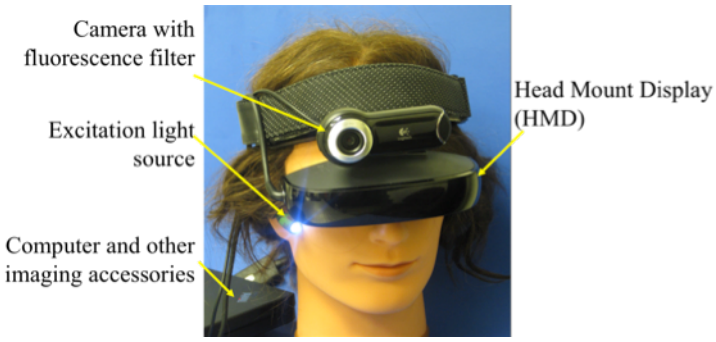

Fig. 3 Prototypical surgical goggle system

Excitation and background light illuminate by turns while the camera takes fluorescence and background image. The fluorescence image is acquired by thresholding and then superimposed on the grayscale background image. The whole process is showed in Fig. 4.

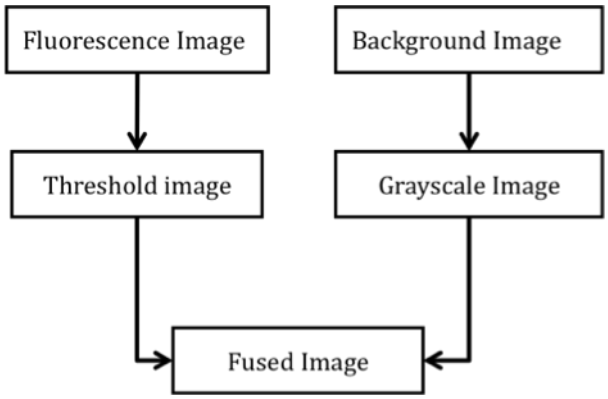

Fig. 4 Schematic of image processing

4. Benchtop test design

To test the feasibility and reliability of the system, we prepare a tissue simulation phantom with embedded simulation tumor. The tissue is made of gelatin mixed with red non-fluorescence dye. To better mimic disseminated characteristic of human tissue, we add white powder. Texas Red (central excitation wavelength 596, emission 613) blended with gelatin is used to simulate antibody-conjugated fluorophore accumulated on the tumor. 4 non-fluorescence simulation benign tissues are placed around the tumor. Another layer of tissue is placed and covered the top. The configuration of the phantom is showed in Fig. 5.

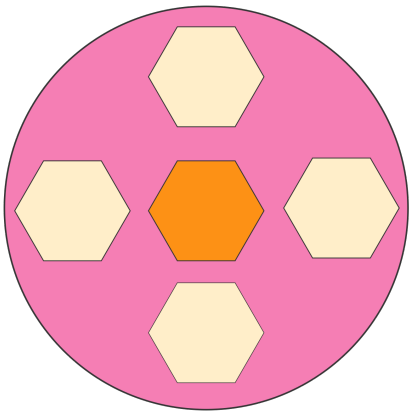
Fig. 5 Configuration of the phantom

A 610nm long pass filter is integrated with the camera. In test set I, excitation lights source is a green LED (central wavelength 535) with Lambert beam pattern since it can provide robust fluorescence without overwhelming the light filter. A red LED with central wavelength at 635nm and Lambert beam pattern is used as background light. Image can be acquired by handholding the excitation light and scanning the phantom.

In test sets II, excitation lightsource is by a handheld green laser pointer (central wavelength 535 nm). In this set, more homogeneous illumination can be reached.

5. Animal model

The surgical goggle system discussed above is validated on the animal model. A xenograft colonel cancer mouse is generated from human colon cancer cells LS174T since they have high levels of TAG-72. To prepare these models, 10^7 tumor cells in matrigel medium (100μl) is injected subcutaneously into NU/NU nude mice. Tumor is allowed to grow for two weeks, with tumor volumes measured twice per week. IV injection of HuCC49-Cy is performed one day before surgery. After 3 days inspection, tumor is surgical removed with image-guidance by the proposed surgical goggle and with the direct injection of antibody-fluorophore conjugate. At the end of the experiment, the animal is necropsied and organs is collected for histological and post-resectional imaging in order to compare the outcomes of five subgroups.

Result

1. Benchtop test result

The test set I demonstrates that if the excitation light illuminated the tumor, the output image will show a red area imposed on the background, indicating the tumor with fluorescence. If the excitation does not illuminate the tumor but benign tissues, only background image will be displayed.

In test set II, the laser pointer will give more stable illumination than that in test set I.

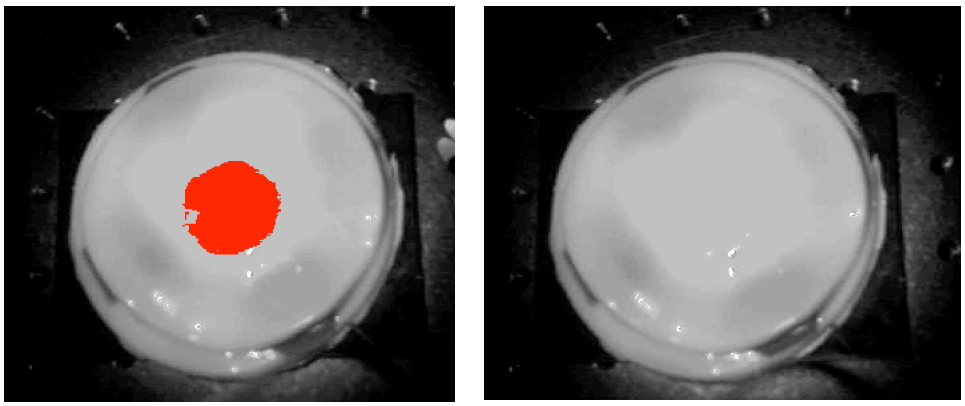

Fig. 6 Bentop test result for test set I. (Left) tumor under illumination; (Right) non-tumor under illumination.

The first image shows the tumor. The second image shows a small spot on benign tumor due to the strong illumination overwhelm the light filter.

2. Animal test result

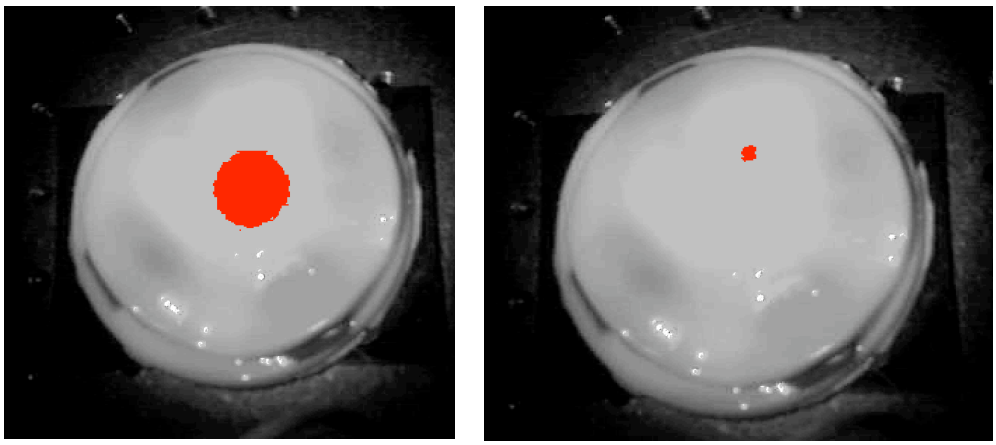

Fig. 7 Benchtop test result for test set II. (Left) tumor under illumination; (Right) non-tumor under illumination.

After IV injection of HuCC49-Cy7, fluorescence images were acquired after 24 hours and 72 hours (excitation: 730nm, emission: 770nm). MuCC49-Cy7 successfully targeted the tumor. From the images in Fig. 8, tumor location and boundary can be directly visualized.

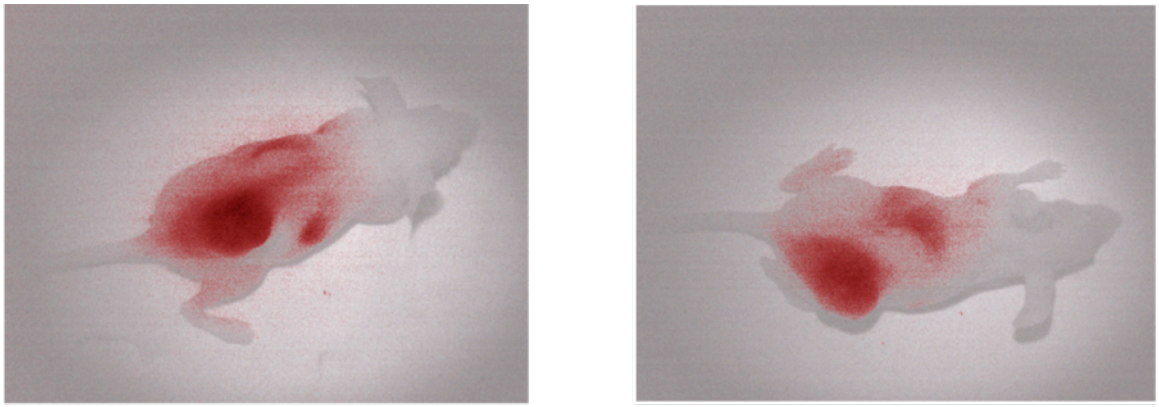

Fig. 8 Sample images that can be visualized from the surgical goggle. (a) The 1st day after IV injection of MuCC49-Cy7. (b) The 3rd day after IV injection of MuCC49-Cy7

Discussion

Disseminate characteristic of tumors makes it a big challenge to the surgeons when performing cancer surgery. However, visualization and properly deal with small foci are of great importance for cancer surgery. We have demonstrated that our targeted fluorescence biomarker can help visualize lesions that are not applicable in traditional white light imaging.

One advantage of our technique is that binding efficiency of HuCC49 is high on colonel cancer, making better fluorescence efficacy. After binding, the tumor cells take up the fluorophore and the rest will go with the circulation, taken and cleared by liver. Those accumulated in the live will not influence the fluorescence in the colonel cancer. Therefore, the distribution of this contrast agent will not influence its application.

There are still potential issues that we are working on. The first is that it usually takes time to collect enough fluorescence. This time will cause lag between image frames. The surgeons may feel dizziness when wearing the surgical goggle. Potential solution can be acquired by our developing fluorescence enhancement technique. By encapsulating fluorescence in a nanoparticle, we can deliver more fluorophore to the target under the condition of the same binding efficiency. This will greatly reduce the exposure time for acquiring fluorescence image and increase frame rate of the fused image.

Also, frame rate can be increase to provide more natural feedback for surgeons. For this purpose, stronger illumination is needed. In our experiment, we use laser pointer for excitation, this will generate stronger fluorescence and reduce exposure time. Also, we can increase the background illumination. Also, we can increase the aperture of the camera, allowing more light to enter the camera.

Another potential issue is caused by heterogeneous illumination of excitation light. Currently, static threshold is used to abstract fluorescence image. Therefore, the distance between the light source and the tissue, the incident angle may cause different illumination and fluorescence strength. To solve this problem, laser pointer will provide better result because laser

is more collimated without scattering beam pattern. Also, our next step is to develop a dynamic algorithm for threshold.

In conclusion, wse believe that surgical goggle is a good concept and is quite potential in the future. We hope that in the future, we and develop a robust, fast and safe intraoperative surgical system for cancer surgery.

Reference

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